

## CLAIMS

### WHAT IS CLAIMED IS:

1. An energy transfer dye comprising:
  - a xanthene donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto;
  - a 4,7-dichlororhodamine acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response thereto; and
  - a non-nucleosidic linker linking the 5- or 6-ring position of the donor dye to the 5- or 6-ring position of the acceptor dye.
2. A fluorescently-labeled reagent comprising a reagent and an energy transfer dye, wherein the energy transfer dye comprises:
  - a xanthene donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto;
  - a 4,7-dichlororhodamine acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response thereto; and
  - a non-nucleosidic linker linking the 5- or 6-ring position of the donor dye to the 5- or 6-ring position of the acceptor dye,and wherein the energy transfer dye is covalently linked to the reagent.
3. The fluorescently-labeled reagent of Claim 2 in which the reagent is selected from the group consisting of proteins, polypeptides, polysaccharides, nucleosides/tides, oligonucleotides, oligonucleotide analogs, lipids, solid supports and organic and inorganic polymers.
4. The fluorescently-labeled reagent of Claim 3 in which the reagent is a nucleoside/tide.

5. The fluorescently labeled nucleoside/tide of Claim 4 in which the energy transfer dye is covalently linked to the nucleobase of the nucleoside/tide at the 4'-position of the donor or acceptor dye.

6. The fluorescently labeled nucleoside/tide of Claim 5 in which the energy transfer dye is covalently linked to the nucleobase of the nucleoside/tide by way of an acetylenic amido or alkenic amido linkage.

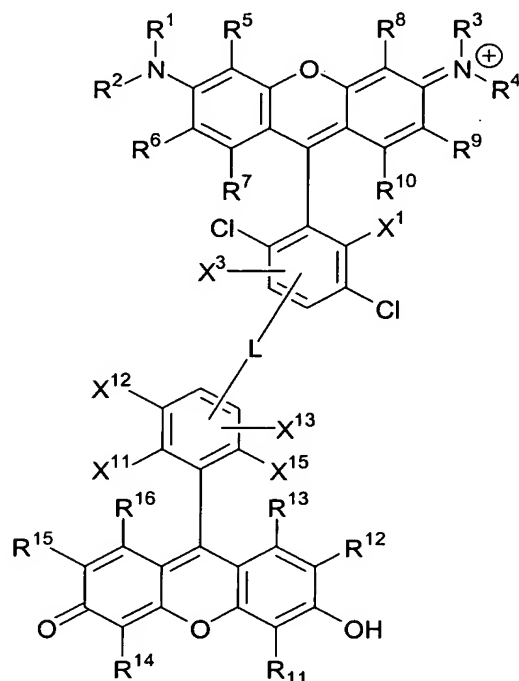
7. The fluorescently labeled nucleoside/tide of Claim 6 in which the acetylenic amido or alkenic amido linkage is selected from -C≡C-CH<sub>2</sub>-NH-C(O)-, 3-amino-1-propyn-1-yl, -C≡C-CH<sub>2</sub>-NH-C(O)-(CH<sub>2</sub>)<sub>5</sub>-C(O)-, -C=CH-C(O)-NH-(CH<sub>2</sub>)<sub>5</sub>-NH-C(O)- and -C≡C-CH<sub>2</sub>-O-CH<sub>2</sub>.CH<sub>2</sub>-NR-, where R is hydrogen, a protecting group or alkyl.

8. The fluorescently-labeled nucleoside/tide of Claim 4 in which the donor dye is a fluorescein.

9. The fluorescently-labeled nucleoside/tide of Claim 4 in which the linker has a backbone that is less than 9 atoms in length.

10. The fluorescently-labeled nucleoside/tide of Claim 4 in which the linker comprises a functional group selected from an alkene, a diene, an alkyne, a five membered ring having at least one unsaturated bond, a six membered ring having at least one unsaturated bond and a fused ring structure.

11. The fluorescently-labeled nucleoside/tide of Claim 4 in which the energy transfer dye comprises the structure:



wherein:

$R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are each, independently of one another, selected from hydrogen and alkyl, or alternatively  $R^1$  and  $R^5$ ,  $R^2$  and  $R^6$ ,  $R^3$  and  $R^8$  and/or  $R^4$  and  $R^9$  may be taken together with the atoms to which they are bonded to form a 5, 6 or 7-membered ring;

$R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$  and  $R^{10}$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, alkoxy, phenyl and substituted phenyl, or alternatively,  $R^6$  and  $R^7$  and/or  $R^9$  and  $R^{10}$  may be taken together with the atoms to which they are bonded to form a benzo group;

$X^1$  and  $X^3$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile and alkoxy;

L is the linker linking the donor and acceptor dyes;

$R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$  and  $R^{16}$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, alkoxy, phenyl and substituted phenyl, or alternatively,  $R^{12}$  and  $R^{13}$  and/or  $R^{15}$  and  $R^{16}$  may be taken together with the atoms to which they are bonded to form a benzo group;

$X^{11}$ ,  $X^{12}$ ,  $X^{13}$  and  $X^{15}$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile and alkoxy; and

$R^8$  or  $R^{14}$  comprises the attachment to the nucleoside/tide.

12. The fluorescently labeled nucleoside/tide of Claim 11 in which the nucleoside/tide is a 2'-deoxyribonucleoside.

13. The fluorescently labeled nucleoside/tide of Claim 11 in which the nucleoside/tide is a 2'-deoxyribonucleotide.

14. The fluorescently labeled nucleoside/tide of Claim 13 in which the 2'-deoxyribonucleotide is a 2'-deoxyribonucleoside-5'-triphosphate.

15. The fluorescently labeled nucleoside/tide of Claim 11 in which the nucleoside/tide is a terminating nucleotide.

16. The fluorescently labeled nucleoside/tide of Claim 15 in which the terminating nucleotide is a 2',3'-dideoxynucleoside-5'-triphosphate.

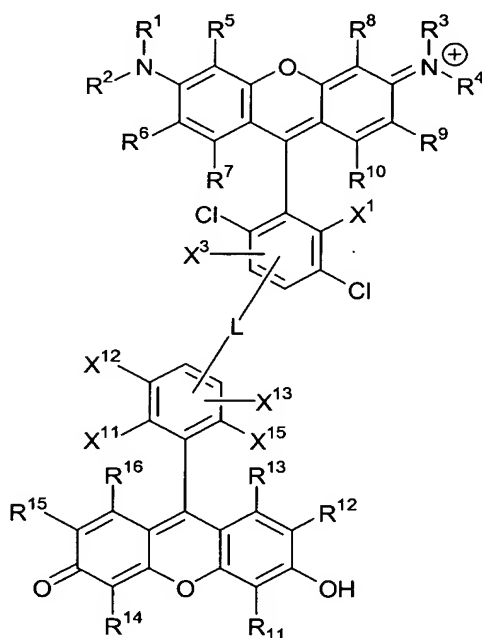
17. A fluorescently-labeled reagent of Claim 3 in which the reagent is an oligonucleotide or oligonucleotide analog.

18. The fluorescently-labeled oligonucleotide or analog of Claim 17 in which the energy transfer dye is attached to the 5'-terminus of the oligonucleotide or analog.

19. The fluorescently-labeled oligonucleotide or analog of Claim 17 in which the energy transfer dye is attached to the 3'-terminus of the oligonucleotide or analog.

20. The fluorescently-labeled oligonucleotide or analog of Claim 17 in which the energy transfer dye is attached to a nucleobase of the oligonucleotide or analog.

21. The fluorescently-labeled oligonucleotide or analog of Claim 17 in which the energy transfer dye comprises the structure:



wherein:

$R^1$ ,  $R^2$ ,  $R^3$  and  $R^4$  are each, independently of one another, selected from hydrogen and alkyl, or alternatively  $R^1$  and  $R^5$ ,  $R^2$  and  $R^6$ ,  $R^3$  and  $R^8$  and/or  $R^4$  and  $R^9$  may be taken together with the atoms to which they are bonded to form a 5, 6 or 7-membered ring;

$R^5$ ,  $R^6$ ,  $R^7$ ,  $R^8$ ,  $R^9$  and  $R^{10}$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, alkoxy,

phenyl and substituted phenyl, or alternatively,  $R^6$  and  $R^7$  and/or  $R^9$  and  $R^{10}$  may be taken together with the atoms to which they are bonded to form a benzo group;

$X^1$  and  $X^3$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile and alkoxy;

L is the linker linking the donor and acceptor dyes;

$R^{11}$ ,  $R^{12}$ ,  $R^{13}$ ,  $R^{14}$ ,  $R^{15}$  and  $R^{16}$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile, alkoxy, phenyl and substituted phenyl, or alternatively,  $R^{12}$  and  $R^{13}$  and/or  $R^{15}$  and  $R^{16}$  may be taken together with the atoms to which they are bonded to form a benzo group;

$X^{11}$ ,  $X^{12}$ ,  $X^{13}$  and  $X^{15}$  are each, independently of one another, selected from hydrogen, fluorine, chlorine, bromine, iodine, carboxyl, alkyl, alkene, alkyne, sulfonate, sulfone, amino, ammonium, amido, nitrile and alkoxy; and

$R^8$  or  $R^{14}$  comprises the attachment to the oligonucleotide or analog.

22. A method of forming a fluorescently labeled oligonucleotide, comprising the step of enzymatically extending an oligonucleotide primer in the presence of a template nucleic acid and nucleotide triphosphates, wherein the oligonucleotide primer or at least one of the nucleotide triphosphates is labeled with an energy transfer dye comprising:

(i) a xanthene donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto;

(ii) a 4,7-dichlororhodamine acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response thereto; and

(iii) a non-nucleosidic linker linking the 5- or 6-ring position of the donor dye to the 5- or 6-ring position of the acceptor dye,

wherein the energy transfer dye is attached to the oligonucleotide primer or the at least one nucleotide triphosphate at the 4'-position of the donor or acceptor dye.

23. The method of Claim 22 in which the oligonucleotide primer is labeled with the energy transfer dye.

24. The method of Claim 22 in which the at least one nucleotide triphosphate is labeled with the energy transfer dye.

25. A method of analyzing a nucleic acid, comprising the steps of:  
forming a plurality of differently sized fluorescently labeled extended oligonucleotide primers;  
separating the fluorescently labeled extended oligonucleotide primers based upon their sizes; and  
detecting the fluorescently labeled extended primers based upon their fluorescence, wherein the fluorescent label of at least one of the fluorescently labeled extended oligonucleotide primers is a fluorescent energy transfer dye comprising:

(i) a xanthene donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto;

(ii) a 4,7-dichlororhodamine acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response thereto; and

(iii) a non-nucleosidic linker linking the 5- or 6-ring position of the donor dye to the 5- or 6-ring position of the acceptor dye,

wherein the energy transfer dye is linked to the extended oligonucleotide primer at the 4'-position of the donor dye or the acceptor dye.

26. The method of Claim 25 in which the plurality of differently sized fluorescently labeled extended oligonucleotide primers are formed by enzymatically extending an oligonucleotide primer comprising the energy transfer dye in the presence of a template nucleic acid, nucleotide triphosphates

and at least one terminating nucleotide such that the primer is extended until the terminating nucleotide is incorporated into the extended primer.

27. The method of Claim 25 in which the plurality of differently sized fluorescently labeled extended oligonucleotide primers are formed by enzymatically extending an oligonucleotide primer in the presence of a template nucleic acid, nucleotide triphosphates and at least one terminating nucleotide comprising the energy transfer dye such that the primer is extended until the terminating nucleotide is incorporated into the extended primer.

28. The method of Claim 27 in which the enzymatic extension is carried out in the presence of four different terminating nucleotides, each of which terminates primer extension at a different template nucleotide, and wherein each of the different terminating nucleotides includes a different, spectrally resolvable fluorescent dye, at least one of which is the energy transfer dye.

29. The method of Claim 28 in which each different terminating nucleotide includes a different, spectrally resolvable energy transfer dye comprising:

(i) a xanthene donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto;

(ii) a 4,7-dichlororhodamine acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response thereto; and

(iii) a non-nucleosidic linker linking the 5- or 6-ring position of the donor dye to the 5- or 6-ring position of the acceptor dye,

wherein the energy transfer dye is linked to the terminating nucleotide at the 4'-position of the donor dye or the acceptor dye.

30. The method of Claim 28 further comprising the step of determining the sequence of the nucleic acid.

31. A kit for sequencing a nucleic acid, comprising a set of four different terminating nucleotides, each of which terminates a template-dependent primer



extension reaction at a different template nucleotide and comprises a fluorescent label capable of producing a detectable, spectrally resolvable fluorescent signal, wherein at least one of the fluorescent labels is an energy transfer dye comprising:

a xanthene donor dye capable of absorbing light at a first wavelength and emitting excitation energy in response thereto;

a 4,7-dichlororhodamine acceptor dye capable of absorbing the excitation energy emitted by the donor dye and fluorescing at a second wavelength in response thereto; and

a non-nucleosidic linker linking the 5- or 6-ring position of the donor dye to the 5- or 6-ring position of the acceptor dye,

wherein the energy transfer dye is linked to the terminating nucleotide at the 4'-position of the donor dye or the acceptor dye.